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Guidance for Industry

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U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES, FOOD AND DRUG ADMINISTRATION

GUIDANCE*
TOPICAL DERMATOLOGIC CORTICOSTEROIDS: *IN VIVO* BIOEQUIVALENCE

Issue Date: 2 June 1995

I. INTRODUCTION

This *Guidance* provides recommendations to pharmaceutical sponsors on methods to document *in vivo* bioequivalence of topical dermatologic corticosteroids, hereinafter referred to as topical corticosteroids. The *Guidance* becomes effective 2 June 1995. Any investigations initiated after that date should generally conform to the recommendations of the *Guidance*. The *Guidance* utilizes a pharmacodynamic approach, based on an update of the Stoughton-McKenzie vasoconstrictor bioassay, to assess bioequivalence of topical corticosteroids. The method utilizes a duration of exposure (dose duration) approach to control the dose of topical corticosteroid that is delivered. The proposed methodology includes a pilot dose duration-response study to determine the appropriate dose duration for use in the pivotal study, followed by the pivotal *in vivo* bioequivalence study incorporating replicate design and documentation of acceptable individual subject dose duration-response. As with all bioanalytical methods, this pharmacodynamic bioassay will require careful validation on the part of pharmaceutical sponsors.

Potent topical corticosteroid products may suppress the hypothalamic-pituitary-adrenal (HPA) axis. In the past, when *in vivo* bioequivalence of such products was documented using the single time point Stoughton-McKenzie study design, the Office of Generic Drugs (OGD) required an HPA axis suppression test when test and reference formulations were significantly different. Products documented to be bioequivalent using this *Guidance* will not be required to submit HPA axis suppression test data.

The 1 July 1992 *Interim Guidance, Topical Corticosteroids: In Vivo Bioequivalence and In Vitro Release Methods*[1] included dermatopharmacokinetic (skin stripping) studies and *in vitro* release studies. The agency currently has insufficient data to recommend skin stripping methods to document bioequivalence of topical corticosteroids. However, this methodology for documentation of bioequivalence may be used if appropriate validation data are provided. At the present time, OGD will not require *in vitro* release data to support approval of ANDA's for topical corticosteroids. Following future recommendations of the Scale-Up and Post-Approval Changes for Semisolids (SUPAC-SS) Working Group, OGD may recommend the submission of *in vitro* release

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* This statement has been prepared by the Division of Bioequivalence in the Office of Generic Drugs, with the participation of the Division of Topical Drug Products in the Office of Drug Evaluation II and the Division of Biometrics in the Office of Epidemiology and Biostatistics. It is an informal communication under 21 CFR 10.90(b)(9) that represents the best judgment of the two reviewing divisions at this time. This statement does not necessarily represent the formal position of the Center for Drug Evaluation and Research, Food and Drug Administration, and does not bind or otherwise obligate the Center for Drug Evaluation and Research. For information about this guidance, contact the Division of Bioequivalence, 7500 Standish Place, Metro Park North, Rockville, MD 20855 (Phone: 301-594-2290; Fax: 301-594-0181).

data to support waiver of *in vivo* bioequivalence of the lower strength(s) of topical corticosteroid products, scale-up of production batches, and approval of formulation, process, and site changes in the absence of *in vivo* data. Future use of these data as a quality control tool is also envisioned.

The *Guidance* has been prepared by staff of the Division of Bioequivalence (HFD-650), with the participation of staff of the Division of Topical Drug Products (HFD-540) and the Division of Biometrics (HFD-710). It is a general guidance intended to apply to topical corticosteroids of all potency groups. Because dose duration-response characteristics may vary with the particular drug of interest, as well as with study conditions, the *Guidance* encourages the performance of a pilot study to define appropriate parameters for the pivotal study. Staff members of HFD-650 are available to work with pharmaceutical sponsors in the design of specific studies to meet the recommendations of the *Guidance*.

II. BACKGROUND

The determination of bioequivalence of two solid oral dosage forms generally rests on a comparison of drug and/or metabolite concentrations in an accessible biologic fluid, such as blood or urine, after administration of a single or multiple doses of each drug product to healthy volunteers. In the absence of this methodology, the Food and Drug Administration may, through provisions of the Food, Drug and Cosmetic Act and implementing regulations (21 CFR § 320), rely on other *in vivo* and *in vitro* methods to assess bioequivalence. In descending order of preference within the Office of Generic Drugs, these methods include: 1) pharmacodynamic effect studies; 2) clinical trials, 3) *in vivo* animal studies; and 4) *in vitro* studies. Although the methods in the latter two categories are acceptable from a statutory/regulatory standpoint, the Division of Bioequivalence in OGD historically has relied only on *in vivo* pharmacodynamic or clinical studies to assess bioequivalence of drug products that do not produce measurable concentrations of drug or metabolite in an accessible biological fluid. Clinical trials generally require large numbers of subjects and often lack sensitivity. In contrast, pharmacodynamic effect studies offer the possibility of developing acceptable bioequivalence data in a relatively small number of subjects.

For many years, the Division of Bioequivalence has relied on pharmacodynamic effect methodology to approve generic topical corticosteroid drug products. The approach is based on the property of corticosteroids to produce blanching or vasoconstriction in the microvasculature of the skin. This property presumably relates to the amount of drug entering the skin[2] and thus becomes a possible basis for the comparison of drug delivery from two potentially equivalent topical corticosteroid formulations. Development of the methodology is attributed to Dr. R.B. Stoughton and Dr. A.W. McKenzie, who initially developed it as a means to assess potency of different topical corticosteroids.[3] Subsequently it was applied by pharmaceutical manufacturers and accepted by the Food and Drug Administration as a means of assessing bioavailability and bioequivalence. In these and other applications, it is referred to variously as the Stoughton-McKenzie test, the vasoconstrictor assay, or the skin blanching assay. Although there are many forms of the vasoconstrictor assay, the general method is

based on topical application of a corticosteroid-containing formulation for a period of 6 to 16 hours in healthy human subjects, followed by visual estimation by a trained, blinded observer of the degree of blanching on a multiple unit scale (0 - 3 or 0 - 4), at a single time point, usually two hours, after removal of the formulation. Most of the currently available generic topical corticosteroids have been approved on the basis of the vasoconstrictor assay as described in this paragraph, or a variant thereof, by OGD after consultation with the Division of Topical Drug Products. These studies were conducted prior to 1 July 1992.

The *Guidance* suggests conducting two *in vivo* studies - a pilot dose duration-response study and a pivotal *in vivo* bioequivalence study comparing test and reference products. The pilot study characterizes the dose duration-response relationship for the drug in terms of the E_{max} model (Section III) and is conducted solely with the reference listed drug (RLD). The dose duration method as recommended in this guidance for documentation of bioequivalence is based on three dose durations: ED_{50}, D_1 and D_2. The comparison of test and reference products in the pivotal study is conducted at a dose duration approximately equal to the population ED_{50} determined in the pilot study. Sensitivity in the pivotal study is established through dosing of the RLD calibrators at two dose durations, D_1 (the shorter dose duration calibrator) and D_2 (the longer dose duration calibrator). The guidance recommends that D_1 equal approximately 0.5 times ED_{50}, and D_2 equal approximately 2 times ED_{50} determined from the pilot study. Because each subject becomes a 'detector' in the study, only the data of those subjects whose D_2/D_1 ratio of pharmacodynamic responses meets a specified minimum value may be included in the data and statistical analyses supporting *in vivo* bioequivalence. The proposed methodology is more fully explained in subsequent sections of the guidance.

III. PHARMACODYNAMIC EFFECT STUDIES: THE VASOCONSTRICTOR ASSAY

Regulatory concerns regarding the vasoconstrictor assay, as it is currently performed, focus on two interrelated aspects of the methodology: 1) its validation and standardization as a bioassay; and 2) the use of a trained observer to measure vasoconstrictor response.

A. Validation and Standardization

Application of the vasoconstrictor assay to a determination of bioequivalence of a topical corticosteroid rests on the assumption that the vasoconstrictor properties of a topical corticosteroid can be utilized to establish a standard, validated bioassay. Development and validation of any assay, including a bioassay, involves certain documentation. Elements of this documentation that are of interest to scientists in the Center for Drug Evaluation and Research (CDER) were discussed at a December 1990 workshop cosponsored by the Food and Drug Administration, the American Association of Pharmaceutical Scientists, the Federation Internationale Pharmaceutique, the Canadian Health Protection Branch, and the Association of Official Analytical Chemists. A summary of the conclusions of this workshop has been published.[4]

In considering the following sections, the reader may find it useful to compare assay validation for a standard HPLC or GLC assay with validation for the vasoconstrictor bioassay. The latter method substitutes an observed pharmacodynamic response to an amount of drug, in this instance the vasoconstrictor response to a topical corticosteroid, for the detector response of an HPLC or GLC to a known amount of drug. Whereas only one instrument and detector are used in a standard blood or urine level assay, each subject in a pharmacodynamic bioassay study becomes the 'detector' responding to a known or unknown amount of drug. Despite many fundamental differences between a standard blood or urine level assay and a bioassay, many of the principles regarding standardization and validation are comparable. Several of these issues are discussed in the following sections.

1. Linearity

Understanding of basic pharmacodynamic relationships between either dose or concentration and a pharmacodynamic response of interest has expanded considerably over the last 15 years.[5] Application of this knowledge to the vasoconstrictor bioassay relates to an assessment of linearity. Although various models are available to express a relationship between drug dose and effect, one that may be especially useful to the vasoconstrictor bioassay is the E_{max} model, or the related sigmoid E_{max} model, which describes some measure of effect (E) in terms of a baseline effect (E_0), a maximal effect (E_{max}) and a dose (D) at which the effect is half-maximal (ED_{50}):

$$E = E_0 + \frac{E_{max} \times D}{ED_{50} + D}$$

The *in vivo* vasoconstrictor response generally approaches a maximum. Therefore, a primary issue requiring resolution in the application of the vasoconstrictor assay to assess bioequivalence is whether, at the strengths of the formulations to be tested in the assay, the capacity of the microvasculature of the skin to respond linearly has been exceeded. At relatively high strengths of a topical corticosteroid, minimal change in the vasoconstrictor response may occur, irrespective of increments in dose duration. At relatively low strengths of a topical corticosteroid, the question becomes one of determining the minimal dose that will produce a reliable and reproducible vasoconstrictor response. These questions are analogous to those confronted in the validation of an assay for drug levels in blood or urine, namely, to define the standard curve of an assay and the lower limit of sensitivity. Development and validation of a dose-response standard curve is essential to estimation of ED_{50}, D_1 and D_2.

In standard analytical methods validation, establishing linearity in detector response is necessary. Linearity in response is also desirable in the development of a vasoconstrictor assay. Because the intended generic and reference commercial formulations may be marketed at strengths that produce responses on the flat portion of the dose-response curve, the assay must be optimized to assure that the products are compared in the linear portion of the curve. Development of a dose-response relationship for a topical corticosteroid relies on some reliable way to administer a predetermined dose of the drug product to the skin. In the *Interim Guidance*,[1] three methods were postulated to be acceptable ways of reliably delivering a dose of topical corticosteroid: 1) the dose duration method; 2) the dilution method; and 3) the area method. Both the dose duration method and the dilution method showed promising results in agency sponsored studies.[6] From a formulation viewpoint, the dilution method is impractical, thus CDER scientists believe that the dose duration method is the most suitable for documentation of bioequivalence of topical corticosteroids. Development of a dose duration-response relationship for a topical corticosteroid will indicate points in the effect-time relationship at which the vasoconstrictor response becomes insensitive. In principle, the time course of response should be followed to return to baseline to insure that at each dose duration, the maximal pharmacodynamic response is observed.

2. Accuracy, Precision and Sensitivity

Development of methodology to establish the accuracy, precision, and sensitivity of a bioassay for a topical corticosteroid should be coincident with the development of an acceptable standard curve for the vasoconstrictor assay. This information, as well as the standard curve, should be developed for each study population. As with a standard blood or urine assay, this information will be developed through the use of untreated controls and calibrators containing the topical corticosteroid of interest. Replication of the untreated controls and calibrators will allow estimation of coefficients of variation. Just as a calibrator in a standard HPLC or GLC assay involves measurement of the detector response to a known concentration of drug substance, the calibrator for a pharmacodynamic topical corticosteroid bioassay, based on the dose duration method, involves application of a standard strength of a topical corticosteroid product for different periods of time.

B. Measurement of Vasoconstrictor Response

In an era with increasingly sophisticated methods to detect changes in light, temperature, pressure, and other physical and chemical changes, the use of a human observer to assess the magnitude of a pharmacodynamic effect becomes increasingly inadequate. Application of a commercially available chromameter (or colorimeter; e.g., Chroma Meter 200 or 300 model series,

Minolta) to detect erythema offers the possibility of replacing subjective visual scoring in the vasoconstrictor assay with objective, quantifiable measurements.[7,8] The Division of Bioequivalence currently considers the use of a chromameter to be applicable to bioequivalence studies based on the vasoconstrictor assay, and therefore recommends that pharmaceutical sponsors incorporate the use of a chromameter into their study designs. However, with acceptable validation, which includes establishing the correlation between chromameter measurements and visual estimation data, sponsors may rely on visual estimation of the degree of vasoconstriction.

C. Some Conclusions from Agency-Sponsored Studies

Results of vasoconstrictor assays conducted under agency contract have led OGD to conclude the following:

1. The chromameter possesses greater sensitivity to skin blanching than does visual estimation,

2. Skin blanching response measured over two consecutive 24 hour periods (48 hours) appears to follow a circadian pattern, possibly the result of a circadian pattern in plasma cortisol levels.[9,10] AUEC data through at least 24 hours from time of drug product removal or drug product application [Section IV(I)(9)], appear acceptable for bioequivalence comparisons, and

3. For baseline-adjusted, untreated control site-corrected AUEC data based on chromameter measurements, these studies suggest there is no strong indication of:

   a. a difference in response between left and right arms, or

   b. a location effect on the arm when skin sites are no closer than 3 - 4 cm to the antecubital fossa or to the wrist.

   Using the experimental design recommended in Section V(G)(2), in which the application pattern on each arm is complementary, e.g., T is complementary to R, the impact of such effects, should they occur, is minimized.

IV. PILOT DOSE DURATION-RESPONSE STUDY

The purpose of the pilot study is to determine the dose duration-response relationship of the topical corticosteroid to be studied in the pivotal *in vivo* bioequivalence study. The study is analogous to developing a standard curve in the assay of a drug in a biologic fluid matrix. The outcome of the pilot study provides the dose duration-response information necessary to determine the parameters ED_{50}, D_1, and D_2 to be used in the firm's pivotal *in vivo* bioequivalence study, and an estimation of the

proportion of subjects expected to meet the minimum D_2/D_1 ratio of AUEC values in the pivotal study. Because outcome of a pilot study may be a function of study conditions, including among other factors subject population characteristics, methodology used to assess skin blanching, and amount of drug product applied, this *Guidance* strongly encourages the performance of a pilot study by each study site for each reference listed drug (RLD) under investigation. Refer to Section IV(J)(3) regarding consultation with the Division of Bioequivalence concerning conduct and/or outcome of a pilot study.

A. Study Design and Analysis

1. Dose duration-response study based on RLD only, with randomization of dose duration skin sites.

2. Dose durations from 0.25 to 6.0 hours, plus untreated control sites on each arm to enable correction of active drug skin sites for color changes during the study unrelated to drug exposure. Because the vehicle corresponding to the RLD is not generally available, untreated control sites refer to untreated areas of skin, not to areas of skin to which vehicle has been applied.

3. Chromameter measurement of the pharmacodynamic response to the topical corticosteroid at various time periods, rather than a single time point measurement, following each dose duration application and removal.

4. Dose duration-response data should be modeled using either a nonlinear mixed effect modeling method or a naive pooled data method to determine the population ED_{50} value which will serve as the approximate dose duration for the bioequivalence comparison in the pivotal study.

5. Twelve subjects.

6. For products marketed in multiple strengths, the pilot and pivotal studies should be conducted on the high strength product.**

** Waiver of *in vivo* bioequivalence for lower strengths of a topical dermatologic corticosteroid product will be considered based on acceptable *in vivo* bioequivalence data for the high strength product and comparative formulation data which meet the qualitative sameness (Q_1) and quantitative sameness (Q_2) requirements of the Office of Generic Drugs' Inactive Ingredients Policy for the specific lower strength product relative to the comparable strength innovator product. If the inactive ingredients of the lower strength product do not meet the Q_1 and Q_2 requirements relative to the comparable strength innovator product, waiver will be considered with an explanation.

B. Subject Inclusion Criteria

1. Healthy subjects.

2. Subjects demonstrating adequate vasoconstriction to topical corticosteroids, i.e., 'responders' [Section IV(E)].

3. Written informed consent.

4. Willingness to follow study restrictions.

C. Subject Exclusion Criteria

1. Clinically significant hypertension or circulatory disease.

2. Individuals smoking within one week of study.

3. Caffeine intake greater than 500 mg per day prior to or during the study. (A cup of coffee contains about 85 mg of caffeine).

4. Clinically significant history of alcoholism or drug abuse.

5. Use of topical dermatologic drug therapy on ventral forearms, including prior dosing of a topical corticosteroid in a pharmacodynamic study to a particular skin site, within one month prior to the study.

6. Adverse reactions to topical or systemic corticosteroids.

7. Any current or past medical condition, including active dermatitis or any other dermatologic condition, which might significantly affect pharmacodynamic response to the administered drug.

8. Persons who would require shaving ventral forearms to insure consistent dose on skin surface.

9. Use of any vasoactive (constrictor or dilator) medication, prescription or OTC, that could modulate blood flow. Examples of such drugs include nitroglycerin, antihypertensives, antihistamines, NSAID's, aspirin, and OTC cough/cold products containing antihistamines and/or either phenypropanolamine or phentolamine.

10. Any obvious difference in skin color between arms.

D. Study Restrictions

1. No exercise with either arm, and no strenuous exercise overall, for study duration.

2. No bathing or showering during the periods of drug application and assessment of skin blanching.

3. No use of creams, emollients, or similar products to forearms for 24 hours prior to and throughout the study.

E. Subject Screening for Response

1. Inclusion of 'nonresponders' reduces the ability of a study to detect true differences between test and reference products, should they exist. Therefore, for both the pilot dose duration-response study and the pivotal bioequivalence study, only 'responders,' i.e., subjects who have the capacity to vasoconstrict when dosed with the RLD used in the study, should be included.

2. In this *Guidance*, a 'responder' is defined as a subject who shows a response to a single dose duration of the RLD under the same occlusion or nonocclusion conditions used in the pilot and pivotal studies. Quantification of skin blanching in the pilot and pivotal studies by the chromameter is considered to be the most satisfactory. However, because of the discrete multiple unit scale (0 - 3 or 0 - 4) for visual readings, 'responder' status may be based on visual readings. A dose duration of 4 hours (based on a potency group III product,[3] with the results shown in Figure AIII.1), or 6 hours[11] is suggested, with skin blanching assessment 2 hours following drug product removal. A 'responder' shows a visual reading of at least one unit.

3. To conserve skin sites on the forearm for use in the dose duration-response study or bioequivalence study, 'responder' status may be based on studies conducted at sites other than the forearm.

4. Criteria for identification of responders, including dose duration, magnitude of response, and skin site tested, should be included in the study report. Responder status may also be documented from participation in a previous vasoconstrictor assay study.

F. Validation of Assay Precision

Validation of intraspot and interspot precision of the assay methodology should be documented in four to six subjects who meet the criteria and restrictions of Sections IV(B - D). Four untreated control sites on each ventral forearm should be selected. Four chromameter readings of each site should be made within a one hour period.

The validation study documents acceptable precision by the bioequivalence testing firm in utilizing the chromameter for the measurement of skin blanching. The study should be conducted prior to administration of the drug product.

Results should be provided in the pilot study report, if submitted [Section IV)(J)(3)], and in the pivotal *in vivo* bioequivalence study report.

G. Occlusion versus Nonocclusion

Class labeling for topical corticosteroids states that occlusive film may be used for the management of psoriasis or recalcitrant conditions. This statement may be found in labeling of certain products representing all potency groups, although labeling for certain high potency products specifically states that occlusive films are not to be used. Provided occlusion is allowed in the labeling of the specific reference listed drug, the pilot dose duration-response study and pivotal *in vivo* bioequivalence study may be conducted using occlusive film. However, caution must be used, as analyses of pilot studies conducted under agency contract suggest that the ED_{50} (the dose duration to be used in the pivotal study) decreases with increasing topical corticosteroid product potency.[12] Evaluation of dose duration-response requires dose duration data at times less than the ED_{50}. Very short dose durations are difficult to conduct experimentally and tend to produce high variability in response. Thus occlusion may be appropriate only for the lower potency products, e.g., potency groups VI and VII. If occlusion is used for the pilot study, it should also be used for the pivotal study.

H. Methods of Application and Removal

Either of two methods of application and removal may be utilized in the pilot and pivotal studies [Section V(G)(3)]:

1. Staggered application with synchronized removal, in which drug is applied to skin sites at different times and removed at the same time (Appendix I), and;

2. Synchronized application with staggered removal, in which drug is applied to skin sites at the same time and removed at different times (Appendix II).

I. Study Day Activities

1. Subjects should begin the study sessions at approximately the same time each study day (within one hour).

2. Verification by history of adequate washout of excluded drugs.

3. The forearm should be free of any dirt or particulate matter that would interfere with proper drug application or assessment of pharmacodynamic response. Cleansing of the skin is not encouraged because of the possible effects on drug uptake and pharmacodynamic response to the drug product. If necessary, cleansing should be

performed not less than two hours prior to drug product application. If cleansing is performed, this should noted in the study report.

4. Whether the study is conducted under occlusion or nonocclusion conditions, use of a protective, nonocclusive guard to prevent smearing or removal of topical drug product from the skin site. Care should be taken to avoid contact between the guard and any drug product to prevent inadvertent contamination of untreated control sites or other test sites.

5. Skin sites should be no closer than 3 - 4 cm to the antecubital fossa or to the wrist.

6. Application of the RLD to skin sites of identical surface area on the ventral forearms. Suggested dose durations for the pilot study are 0.25, 0.5, 0.75, 1, 1.5, 2, 4 and 6 hours, but may vary depending on the corticosteroid under investigation.

   a. Eight dose durations, i.e., active drug sites, should be equally divided between the two arms.

   b. Amount of drug product, skin site size, and spacing between sites should be determined by the testing laboratory. For reference, some investigators have used doses of 2 - 10 mg of formulation per cm^2 of skin surface area, and 1 cm diameter sites. Sites may be spaced as close as 2.5 cm center-to-center, and may be in a straight line or staggered pattern, depending on skin surface suitability (e.g., vascularity, nevi, etc.) and arm length. If vasoconstrictor effects of two adjacent test sites overlap and the investigator cannot discern between the vasoconstrictor effect at each test site, the subject should be excluded from the data analysis.

7. Use of two untreated control skin sites per arm for studies based on chromameter measurements.

   a. Application to each subject of eight dose durations [Section IV(I)(6)] and four untreated control sites should be randomly assigned among the 12 sites, maintaining two untreated control sites and four dosed sites on each arm (six sites per arm).

   b. Studies based on visual scoring do not require untreated control sites because the reading involves a visual comparison of the treated site to the surrounding skin. Application to each subject of eight dose durations should be randomly assigned between the two arms, maintaining four dosed sites on each arm.

8. Prior to measurement of the pharmacodynamic response at the end of the application period, remaining topical corticosteroid should be gently removed from the skin. This may be accomplished by either of the methods below.

   a. Three consecutive swabbings with dry cotton swabs.

      Suitable for either the staggered application with synchronized removal method, or the synchronized application with staggered removal method.

   b. Washing all skin sites with mild skin cleanser and water, blotting the sites dry with a nonabrasive towel, and allowing to air-dry for at least five minutes prior to evaluation. If after five minutes the subject has any visible cutaneous effects related to washing, a longer waiting period may be necessary.

      i. Suitable for the staggered application with synchronized removal method.

      ii. Cleanse arm surfaces with a minimum amount of skin cleanser, for example one drop of a liquid cleanser worked to a lather in wetted hands, followed by rinsing.

      iii. Examples of mild liquid skin cleansers are Purpose® Gentle Cleansing Wash (Johnson & Johnson), and Cleansing Wash (Neutrogena).

9. Assessment of baseline skin color and skin blanching at each site. Examples of assessment time periods are:

   a. For staggered application with synchronized removal:

      For all dose durations and untreated control sites, baseline readings within one hour prior to drug application of the longest dose duration, and at 0, 2, 4, 6, 19, and 24 hours after drug product removal (Appendix I).

      Time zero equals time of drug product removal.

   b. For synchronized application with staggered removal:

      For all dose durations and untreated control sites, baseline readings within one hour prior to the time of drug application to active drug sites, and 6, 8, 11, 24, and 28 hours after drug product application (Appendix II).

      Time zero equals time of drug product application.

Note: Optimal assessment times for either method of application and removal may require adjustment of these schedules for the particular drug product and study site. For either method, at least one reading should be scheduled between 5 PM and midnight.

J. Data Analyses and Pharmacodynamic Modeling

1. Chromameter Data

a. Adjust the chromameter raw data of each skin blanching response versus time profile (both active drug sites and untreated control sites) for the baseline value at that site. Correct each baseline-adjusted active drug site for the mean of the two baseline-adjusted untreated control sites on the same arm (Tables AIII.1 - AIII.3).

b. Using the trapezoidal rule, compute the area under the effect curve (AUEC) for each baseline-adjusted, untreated control site-corrected dose duration (Tables AIII.3, AIII.4):

i. $AUEC_{(0\text{-}24)}$ for the staggered application with synchronized removal method, or

ii. $AUEC_{(6\text{-}28)}$ for the synchronized application with staggered removal method [based on the dose duration schedule of Section IV(I)(6)]. In the general case, AUEC from the longest dose duration to 28 hours after drug product application is computed.

c. Fitting dose duration-response data by averaging across subjects at each dose duration is not acceptable. Rather, the data should be fit by using all observations of all individual subjects simultaneously. The modeling software should provide ED_{50} and E_{max} values for the data pooled from 12 subjects. The following methods are acceptable:

i. Fitting based on the assumption of a nonlinear mixed effect model (population model) using suitable software (Figure AIII.1). The mixed effect modeling technique accounts for within- and among-subject variability, or

ii. Fitting based on nonlinear least squares regression, pooling individual observations from all subjects (naive pooled data method).

d. Determine the ED_{50} (the dose duration corresponding to half-maximal response).

e. Determine D_1 and D_2 corresponding to approximately one-half ED_{50} and two times ED_{50}, respectively, for use in the pivotal study.*** These values bracket ED_{50}, correspond to approximately 33% and 67% respectively of the maximal response, and represent the sensitive portion of the dose duration-response curve.[5]

2. Visual Data [refer to Section III(B)]

a. Compute the area under the effect curve (AUEC) for each vasoconstriction time profile.

b. Fit the dose duration-response data as described in Section IV(J)(1)(c).

c. Determine the ED_{50}, D_1, and D_2.

3. Consultation with the Division of Bioequivalence

If a sponsor wishes to discuss issues related to assay validation, dose duration-response, or other aspects of its pilot dose duration-response study prior to the conduct of the pivotal *in vivo* bioequivalence study, the sponsor has the option to submit the study data and summary results of the pilot study to the Division of Bioequivalence for review of ED_{50}, D_1, and D_2 values, and the proposed pivotal study protocol. If the pilot study results are submitted, the firm may wish to include all study data, with an explanation accompanying any data not included in the pharmacodynamic analysis.

Sponsors may consider that they have sufficient information about the dose duration-response relationship of the topical corticosteroid under investigation to proceed directly to the pivotal study without conduct of the pilot study. This course of action assumes knowledge of ED_{50}, D_1, and D_2 appropriate to the RLD under study site conditions, which is essential to an acceptable pivotal study. Staff in the Division of Bioequivalence are available to review this information at the request of a sponsor.

*** The observed ED_{50} value may be rounded by up to 15 minutes to obtain the ED_{50} value used in the pivotal study. In practice, a demonstration of dose duration-response based on D_1 within 0.25 - 0.5 times the observed ED_{50} and D_2 within 2 - 4 times the observed ED_{50} is acceptable. For potent corticosteroids with short ED_{50} values, these recommendations may require adjustment. If so, the Division of Bioequivalence may be consulted.

4. Computer Formatted Data Submission

If the study data and summary results are submitted, a diskette in the ASCII format containing the study data should be submitted with the pilot study. Chromameter raw data; baseline-adjusted data; baseline-adjusted, untreated control site-corrected data; and AUEC data should be arranged in separate files in the format given in Tables AIII.1 - AIII.4.

V. PIVOTAL *IN VIVO* BIOEQUIVALENCE STUDY

The purpose of the pivotal study is to document *in vivo* bioequivalence of the test product to the reference listed drug (RLD). The guidance specifies the minimum dose duration-response ratio which must be met by individual subjects for inclusion in the data analysis. Therefore, a pivotal study may generally be initiated without consultation with the Division of Bioequivalence [Section IV(J)(3)].

A. Study Design

1. Pharmacodynamic bioequivalence study using within-study day replicate single dose durations of test and reference products, and based on the population ED_{50} identified in the pilot study.

2. Individual subject dose duration-response, based upon an acceptable D_2/D_1 ratio of AUEC values of the RLD. The minimum value of the ratio should be 1.25. Success in meeting this dose duration-response criterion will be determined through duplicate dosing of the RLD at D_1, the dose duration equal to approximately 0.5 times the population ED_{50}, and at D_2, the dose duration equal to approximately 2 times the population ED_{50}.

3. Forty to sixty evaluable subjects, i.e., subjects who meet the 'responder' and 'detector' criteria of Sections IV(E) and V(H)(1)(c).

B. Subject Inclusion Criteria

Consult Section IV(B).

C. Subject Exclusion Criteria

Consult Section IV(C).

D. Study Restrictions

Consult Section IV(D).

E. Subject Screening for Response

Consult Section IV(E).

F. Assay Precision

Consult Section IV(F).

G. Study Day Activities

1. Consult Section IV(I), where applicable.

2. Application of dose durations to skin sites on the ventral forearms of each subject should be randomly assigned, maintaining the recommendations described below. Sites may be occluded or nonoccluded, based on the considerations of Section IV(G) and the pilot study results. Untreated control skin sites should also be included for studies based on chromameter measurements. Dose durations and control sites on each arm should include:

   T: the test product at the dose duration corresponding approximately to ED_{50}, as determined with the reference listed drug (RLD) in the pilot study (two sites per arm);

   R: the reference listed drug (RLD) at the same dose duration corresponding approximately to ED_{50} as for the test product T (two sites per arm);

   D_1: the shorter dose duration RLD calibrator (one site per arm);

   D_2: the longer dose duration RLD calibrator (one site per arm); and

   UNT: the untreated control (two sites per arm).

   The total number of testing sites is 16 (eight sites per arm). The eight treatments should be randomized, as noted above. Application patterns on each arm should be complementary, i.e., D_2 is complementary to D_1, R is complementary to T, and UNT is complementary to UNT. As examples, where T is assigned a specific skin site location on one arm, R should be assigned to the corresponding skin site on the other arm. Where UNT is assigned a specific skin site location on one arm, UNT should be assigned to the corresponding skin site on the other arm.

A representative application sequence for a particular subject might be:

ANTECUBITAL FOSSA

Left Arm	Right Arm
D_1	D_2
T	R
UNT	UNT
R	T
UNT	UNT
T	R
D_2	D_1
R	T

WRIST

The specific pattern of skin sites, i.e., medial (ulnar) to lateral (radial), and superior to inferior, should be described by the firm.

3. Either the staggered application with synchronized removal or the synchronized application with staggered removal method, consistent with the methodology used in the pilot study, should be used for D_1, D_2, and ED_{50} dose durations.

4. Examples of time periods for assessment of baseline skin color and skin blanching at each site are:

   a. For staggered application with synchronized removal:

      For all dose durations and untreated control sites, baseline readings within one hour prior to drug application of the longest dose duration; skin blanching readings at 0, 2, 4, 6, 19, and 24 hours after drug product removal. Actual times will depend upon the time of dosing and the topical corticosteroid being studied.

      Time zero equals time of drug product removal.

   b. For synchronized application with staggered removal:

      For all dose durations and untreated control sites, baseline readings within one hour prior to the time of drug application to active drug sites; skin blanching readings at the following times

after drug product application: D_2 (see Note below), 6, 8, 11, 24, and 28 hours. Actual times will depend upon the time of dosing, the topical corticosteroid being studied, and D_2.

Time zero equals time of drug product application.

Note: For example, if D_2 for a specific drug product equals 4 hours, the first post-baseline reading of all skin sites, both active drug sites and untreated control sites, would be at 4 hours. For either method, at least one reading should be scheduled between 5 PM and midnight.

H. Data and Statistical Analyses

1. Data Analysis

a. For the chromameter raw data, adjust each skin blanching response versus time profile (both active drug sites and untreated control sites) for the baseline value at that site. Correct the data of each baseline-adjusted active drug site for the mean of the two baseline-adjusted untreated control sites from the same arm (Tables AIV.1 - AIV.4).

b. Compute AUEC for each baseline-adjusted, untreated control site-corrected dose duration (Tables AIV.3, AIV.5, AIV.6).

i. $AUEC_{(0\text{-}24)}$ for the staggered application with synchronized removal method, or

ii. AUEC from time D_2 to 28 hours, $AUEC_{(D2\text{-}28)}$, for the synchronized application with staggered removal method.

c. Only the data of 'detectors,' i.e., individual subjects whose AUEC values (Table AIV.5) at D_1 and D_2 are both negative and that meet the dose duration-response criterion below, should be included in the data analysis (Tables AIV.6, AV.1). The dose duration-response criterion is:

$$\frac{AUEC \ \ at \ \ D_2}{AUEC \ \ at \ \ D_1} \geq 1.25$$

where:

AUEC at D_2 = 0.5 [AUEC at D_2 (left arm) + AUEC at D_2 (right arm)];

AUEC at D_1 = 0.5 [AUEC at D_1 (left arm) + AUEC at D_1 (right arm)].

d. Only those subjects with a complete data set, i.e., duplicate values of D_1 and D_2, and quadruplicate values of T, R, and UNT, should be included in the data analysis.

e. The bioequivalence comparison should be based on AUEC values computed according to Section V(H)(1)(b) at the dose duration corresponding approximately to ED_{50} [treatments T and R, Section V(G)(2)].

f. All study data, including the data of 'nondetectors,' should be submitted. An explanation (e.g., 'nondetector,' overlap of vasoconstrictor effect due to an adjacent site, etc.) should accompany any data not used in the bioequivalence evaluation.

2. Statistical Analysis

a. The statistical analysis requires the use of untransformed data because AUEC values of treatments T and R, calculated from baseline-adjusted, untreated control site-corrected data, although generally negative, are sometimes positive. The presence of both positive and negative data eliminates the use of conventional statistical transformations. Previously used approximate methods,[13] for example calculating a confidence interval for the difference between test and reference product averages, and dividing these limits by an estimate of the reference product average, are not applicable. Locke's method[14] provides an exact confidence interval from untransformed data.

b. The 90% confidence interval should be calculated for the ratio of the average AUEC response due to the test product (average of four replicates) to the average AUEC response due to the reference product (average of four replicates) using Locke's method. The formulae and a worked example, based on the data of Table AIV.6, are given in Appendix V.

c. The Office of Generic Drugs has not determined at this time the equivalence interval for bioequivalence. The Office recognizes that an equivalence interval wider than 80-125%, as a public standard, may be necessary pending evaluation of data submitted to the agency.

d. The randomization code, indicating the specific skin sites to which each dose duration and control site was assigned, should be submitted with the study report.

3. Computer Formatted Data Submission

A diskette in the ASCII format containing the study data should be submitted with the application. Chromameter raw data; baseline-adjusted data; baseline-adjusted, untreated control site-corrected data; and AUEC data should be arranged in separate files in the format given in Tables AIV.1 - AIV.6.

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REFERENCES

1. Shah VP. Interim guidance, topical corticosteroids: *in vivo* bioequivalence and *in vitro* release methods. Division of Bioequivalence and Division of Anti-Infective Drug Products, Center for Drug Evaluation and Research, Food and Drug Administration, Rockville, MD: 1 July 1992.

2. Pershing LK, Silver BS, Krueger GG, Shah VP, Skelley [sic] JP. Feasibility of measuring the bioavailability of topical betamethasone dipropionate in commercial formulations using drug content in skin and a skin blanching bioassay. Pharm Res 1992;9:45-51.

3. Stoughton RB. Vasoconstrictor assay - specific applications. In: Maibach HI, Surber C, eds. Topical corticosteroids. Basel: Karger, 1992:42-53.

4. Shah VP, Midha KK, Dighe S, et al. Conference report. Analytical methods validation: bioavailability, bioequivalence and pharmacokinetic studies. Pharm Res 1992;9:588-92.

5. Holford NHG, Sheiner LB. Understanding the dose-effect relationship: clinical application of pharmacokinetic-pharmacodynamic models. Clin Pharmacokin 1981; 6:429-53.

6. Pershing LK, Lambert L, Wright ED, Shah VP, Williams RL. Topical 0.050% betamethasone dipropionate: pharmacokinetic and pharmacodynamic dose-response studies in humans. Arch Dermatol 1994;130:740-7.

7. Queille-Roussel C, Poncet M, Schaefer H. Quantification of skin-colour changes induced by topical corticosteroid preparations using the Minolta Chroma Meter. Br J Dermatol 1991;124:264-70.

8. Elsner P. Chromametry: hardware, measuring principles, and standardization of measurements. In: Berardesca E, Elsner P, Maibach HI, eds. Bioengineering of the skin: cutaneous blood flow and erythema. Boca Raton: CRC Press, 1995:247-52.

9. Krieger DT, Allen W, Rizzo F, Krieger HP. Characterization of the normal temporal pattern of plasma corticosteroid levels. J Clin Endocrinol Metab 1971;32:266-84.

10. Pershing LK, Corlett JL, Lambert LD, Poncelet. Circadian activity of topical 0.05% betamethasone dipropionate in human skin *in vivo*. J Invest Dermatol 1994;102:734-9.

11. Smith EW, Meyer E, Haigh JM, Maibach HI. The human skin blanching assay as an indicator of topical corticosteroid bioavailability and potency: an update. In Bronaugh RL, Maibach HI, eds. Percutaneous absorption: mechanisms-methodology-drug delivery. 2nd ed. New York: Marcel Dekker, 1989:443-60.

12. Singh GJP, Lesko LJ, Shah VP, et al. Bioequivalence methodology for dermatologic corticosteroids based on pharmacodynamic modeling [Abstract]. Clin Pharmacol Ther 1995;57:181.

13. Schuirmann DJ. Confidence intervals for the ratio of two means from a crossover study. In: Proceedings of the biopharmaceutical section, American Statistical Association, Washington, DC 1989; 121-6.

14. Locke CS. An exact confidence interval from untransformed data for the ratio of two formulation means. J Pharmacokinet Biopharm 1984;12:649-55.

APPENDIX I

STAGGERED APPLICATION WITH SYNCHRONIZED REMOVAL:
SCHEMATIC FOR A SUGGESTED PILOT STUDY PROTOCOL

Figure AI.1

Baseline (BL) Measurement, Drug Application and Drug Removal

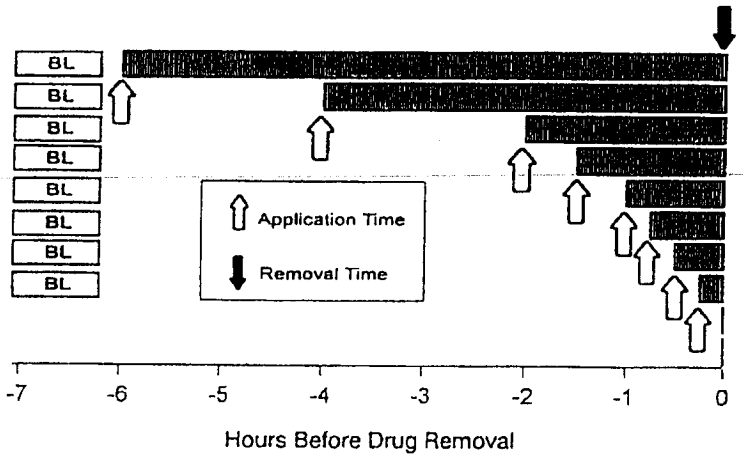
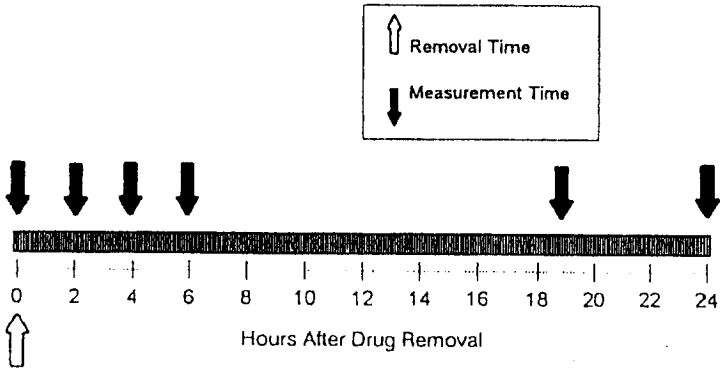


Figure AI.2

Skin Blanching Measurements



APPENDIX II

SYNCHRONIZED APPLICATION WITH STAGGERED REMOVAL:
SCHEMATIC FOR A SUGGESTED PILOT STUDY PROTOCOL

Figure AII.1

Baseline (BL) Measurement, Drug Application and Drug Removal

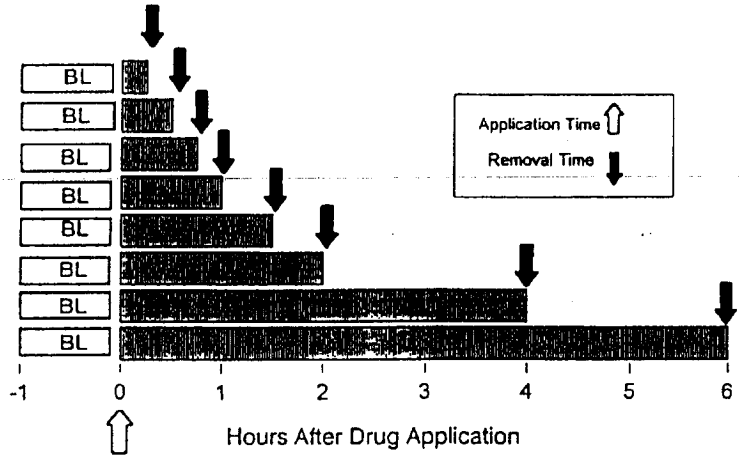
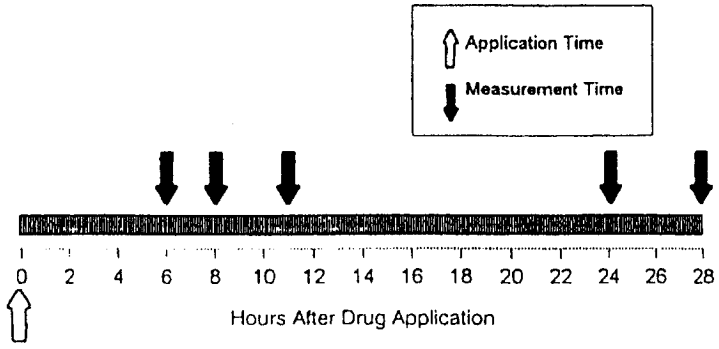


Figure AII.2

Skin Blanching Measurements



APPENDIX III

ANALYSIS OF DATA FROM AN AGENCY-SPONSORED PILOT STUDY: AN EXAMPLE

Development of the recommended study designs in this guidance included the conduct in late 1994 and early 1995 under agency contract [Section III(C)] of small-scale vasoconstrictor assays at two research sites: the University of Utah Health Sciences Center and the University of California, San Diego, School of Medicine. The University of Utah study used the synchronized application with staggered removal method, while the University of California study used the staggered application with synchronized removal method. Each research site conducted a pilot dose duration-response study and a 'pivotal' *in vivo* bioequivalence study, with 12 subjects in each of the four studies. The contract studies differed in several ways from those recommended in this guidance. The contract studies (1) used untreated control sites corresponding to each active drug site, (2) measured skin blanching through 48 hours, and (3) did not replicate the test and reference products on each arm ('pivotal' study).

No preference in methodology is intended by use of data based on the staggered application with synchronized removal method in Appendices III - V to illustrate data analysis. Either method of application and removal is acceptable. Table AIII.1 presents chromameter a-scale raw data of the University of California pilot study for subject 1 through 24 hours. Baseline-adjusted data are presented in Table AIII.2, and baseline-adjusted, untreated control site-corrected data are presented in Table AIII.3. In this example data set, each active drug site was corrected for its corresponding untreated control site. However, the guidance recommends use of only two untreated control sites per arm, and subtracting their average from all active drug sites on that arm. Table AIII.4 presents $AUEC_{(0\text{-}24)}$ data for all subjects and dose durations. The E_{max} model fit to the pooled data is shown in Figure AIII.1.

Table AIII.1 Chroma Meter (Minolta) a-scale readings for a subject

SUB	DD	SITE	BL	Hours after drug removal					
				0	2	4	6	19	24
1	0.25	UNT	9.86	9.99	10.10	9.52	10.03	10.40	9.65
1	0.25	TRT	10.36	9.89	10.38	10.32	10.51	10.86	10.04
1	0.5	UNT	9.27	8.20	9.78	8.54	9.61	9.87	9.59
1	0.5	TRT	9.59	8.77	9.35	9.27	8.78	10.40	9.82
1	0.75	UNT	8.45	8.75	8.24	8.16	8.92	8.43	8.22
1	0.75	TRT	8.46	8.66	8.53	8.04	8.26	8.72	8.56
1	1	UNT	9.00	9.63	8.45	8.03	8.94	9.33	9.66
1	1	TRT	8.52	8.80	8.87	8.53	8.05	8.66	8.21
1	1.5	UNT	9.44	9.39	9.46	9.27	9.92	9.59	9.01
1	1.5	TRT	9.59	9.60	9.99	9.93	9.18	10.23	9.24
1	2	UNT	10.12	10.13	9.50	9.93	9.39	10.95	10.84
1	2	TRT	10.28	10.25	10.68	10.15	10.31	11.46	8.92
1	4	UNT	8.89	8.01	8.78	8.89	9.76	8.48	9.18
1	4	TRT	8.21	8.28	8.36	7.98	7.96	8.15	8.30
1	6	UNT	9.18	9.46	8.79	8.03	9.29	10.11	9.51
1	6	TRT	9.37	9.61	9.30	8.92	9.20	10.16	9.63

Appendix III abbreviations appear on page 28.

Table AIII.2 Baseline-adjusted a-scale data for a subject

SUB	DD	SITE	BL	Hours after drug removal					
				0	2	4	6	19	24
1	0.25	UNT	-	0.13	0.24	-0.34	0.17	0.54	-0.21
1	0.25	TRT	-	-0.47	0.02	-0.04	0.15	0.50	-0.32
1	0.5	UNT	-	-1.07	0.51	-0.73	0.34	0.60	0.32
1	0.5	TRT	-	-0.82	-0.24	-0.32	-0.81	0.81	0.23
1	0.75	UNT	-	0.30	-0.21	-0.29	0.47	-0.02	-0.23
1	0.75	TRT	-	0.20	0.07	-0.42	-0.20	0.26	0.10
1	1	UNT	-	0.63	-0.55	-0.97	-0.06	0.33	0.66
1	1	TRT	-	0.28	0.35	0.01	-0.47	0.14	-0.31
1	1.5	UNT	-	-0.05	0.02	-0.17	0.48	0.15	-0.43
1	1.5	TRT	-	0.01	0.40	0.34	-0.41	0.64	-0.35
1	2	UNT	-	0.01	-0.62	-0.19	-0.73	0.83	0.72
1	2	TRT	-	-0.03	0.40	-0.13	0.03	1.18	-1.36
1	4	UNT	-	-0.88	-0.11	0.00	0.87	-0.41	0.29
1	4	TRT	-	0.07	0.15	-0.23	-0.25	-0.06	0.09
1	6	UNT	-	0.28	-0.39	-1.15	0.11	0.93	0.33
1	6	TRT	-	0.24	-0.07	-0.45	-0.17	0.79	0.26

Table AIII.3 Baseline-adjusted, untreated control site-corrected a-scale data and $AUEC_{(0-24)}$ data for a subject

SUB	DD	SITE	BL	Hours after drug removal						$AUEC_{(0-24)}$*
				0	2	4	6	19	24	
1	0.25	TRT	-	-0.60	-0.22	0.30	-0.02	-0.04	-0.11	-1.23
1	0.5	TRT	-	0.25	-0.75	0.41	-1.15	0.21	-0.09	-7.39
1	0.75	TRT	-	-0.10	0.28	-0.13	-0.67	0.28	0.33	-1.48
1	1	TRT	-	-0.35	0.90	0.98	-0.41	-0.19	-0.97	-3.80
1	1.5	TRT	-	0.06	0.38	0.51	-0.89	0.49	0.08	-0.23
1	2	TRT	-	-0.04	1.02	0.06	0.76	0.35	-2.08	5.77
1	4	TRT	-	0.95	0.26	-0.23	-1.12	0.35	-0.20	-4.74
1	6	TRT	-	-0.04	0.32	0.70	-0.28	-0.14	-0.07	-1.53

* $AUEC_{(0-24)}$ units are baseline-adjusted, untreated control site-corrected a-scale units times hours

Table AIII.4 $AUEC_{(0-24)}$ data of all 12 subjects at each dose duration

DD	Subject Number					
	1	2	3	4	5	6
0.25	-1.23	-0.02	-13.87	-27.27	-10.65	-10.41
0.5	-7.39	-5.13	-15.03	-3.71	7.72	-5.94
0.75	-1.48	-8.92	-18.39	-43.82	-23.42	-2.29
1	-3.80	-24.56	-16.25	-44.39	-20.37	-8.92
1.5	-0.23	-19.21	-15.44	-77.04	-19.95	-20.64
2	5.77	-1.80	-23.74	-66.80	-32.00	-19.52
4	-4.74	-43.07	-24.80	-62.96	-32.81	-8.52
6	-1.53	-41.56	-21.79	-71.60	-61.51	-19.01

DD	Subject Number					
	7	8	9	10	11	12
0.25	4.20	-11.95	-12.36	1.15	-30.03	-7.25
0.5	-12.31	7.45	12.95	-39.45	-39.56	14.73
0.75	1.34	5.95	1.88	-40.68	-61.06	-21.09
1	-18.84	8.78	-43.35	-16.19	-43.58	10.81
1.5	-42.70	1.26	-20.97	6.87	-40.73	0.51
2	-37.29	-48.83	-39.79	10.75	-62.01	-10.51
4	-45.46	-71.77	-57.55	-37.64	-27.82	-14.89
6	-37.24	-8.14	-34.18	-35.01	-33.60	16.14

Abbreviations in Tables AIII.1 - AIII.4

DD: Dose duration in hours
UNT: Untreated control site (no product applied)
TRT: Treated site (topical corticosteroid product applied)
BL: Baseline measurement of skin color, as described in Section IV(I)(9)

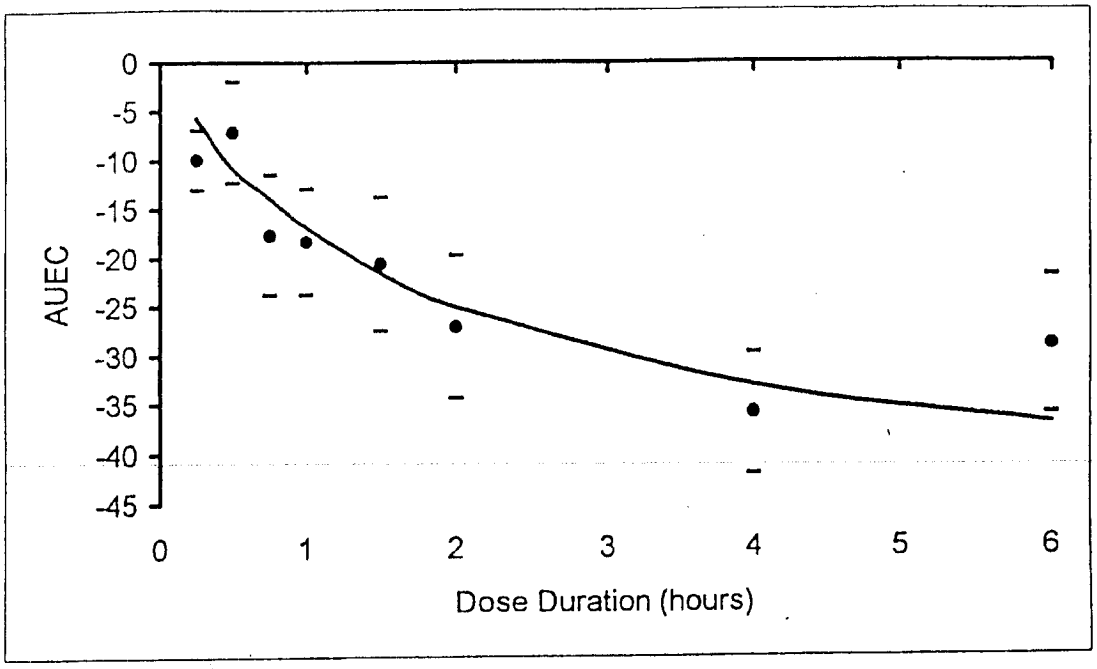


Figure AIII.1 Observed $AUEC_{(0-24)}$ mean (filled circles) and SEM (upper and lower limits), and E_{max} model fitted (solid line) to the pooled dose duration-response data of all 12 subjects in the pilot study.

Note 1: Data are baseline-adjusted and untreated control-site corrected, thus AUEC was set to zero at dose duration equal to zero.

Note 2: The E_{max} model fit (solid line) was determined using a population pharmacokinetic-dynamic data modeling program (P-Pharm, Simed). The fitted population values were:

ED_{50}:	1.89 hours
E_{max}:	-48.80 a-scale units times hours

Note 3: Based on these data, the dose durations selected as the approximate ED_{50} for the comparison of test and reference products, and as D_1 and D_2 in the 'pivotal' *in vivo* bioequivalence study were:

Approximate ED_{50}:	2.0 hours
D_1:	1.0 hour
D_2:	4.0 hours

APPENDIX IV

ANALYSIS OF DATA FROM AN AGENCY-SPONSORED 'PIVOTAL' STUDY: AN EXAMPLE

Appendix IV presents chromameter a-scale data and $AUEC_{(0\text{-}24)}$ data of the University of California 'pivotal' *in vivo* bioequivalence study referred to in Appendix III. In the 'pivotal' study, the bioequivalence comparison is based on the dose duration-response analysis summarized in Figure AIII.1, Note 3. Table AIV.1 presents the a-scale raw data through 24 hours for subject 1. Baseline-adjusted data are presented in Table AIV.2, and baseline-adjusted, untreated control site-corrected data are presented in Table AIV.3. In this example data set, each active drug site was corrected for its corresponding untreated control site. However, the guidance recommends use of only two untreated control sites per arm, and subtracting their average from all active drug sites on that arm. Table AIV.4 presents the baseline-adjusted and untreated control site-corrected a-scale data for test and reference products for all subjects. Table AIV.5 presents right and left arm $AUEC_{(0\text{-}24)}$ data, and the two arm average data, for D_1 and D_2 for all subjects. Table AIV.5 also identifies 'detectors,' i.e., evaluable subjects, defined as those subjects who met the dose duration-response criterion [Section V(H)(1)(c)]. Table AIV.6 presents right and left arm $AUEC_{(0\text{-}24)}$ data, and the two arm average data, for test and reference products for all subjects at a dose duration of 2.0 hours, and highlights the two arm average $AUEC_{(0\text{-}24)}$ data of the 'detectors.' Only the data of 'detectors' are included in the analysis for bioequivalence, as described in Appendix V.

Table AIV.1 Chroma Meter (Minolta) a-scale raw data for a subject

SUB	TRT	ARM	LOC	SITE	BL	Hours after drug removal					
						0	2	4	6	19	24
1	A	R	1	UNT	7.34	7.23	8.09	7.64	7.82	7.68	8.71
1	A	R	1	TRT	7.11	7.86	7.59	5.92	6.23	6.32	7.30
1	B	R	2	UNT	6.18	7.38	7.26	6.85	7.35	7.14	7.87
1	B	R	2	TRT	6.79	6.29	6.12	4.45	5.88	6.01	7.26
1	C	R	3	UNT	6.28	7.32	7.80	6.77	7.75	6.59	7.55
1	C	R	3	TRT	7.78	9.26	9.30	7.42	8.24	7.40	8.59
1	D	R	4	UNT	9.31	10.19	10.61	9.56	10.88	9.52	10.13
1	D	R	4	TRT	7.38	8.22	6.94	5.07	6.98	7.24	7.91
1	C	L	1	UNT	7.62	7.98	7.56	7.48	7.24	6.73	7.49
1	C	L	1	TRT	6.97	5.42	5.39	4.39	4.79	5.76	6.45
1	B	L	2	UNT	7.12	6.32	6.76	6.25	6.74	6.80	7.58
1	B	L	2	TRT	7.46	4.48	4.38	4.11	4.39	6.27	7.25
1	A	L	3	UNT	7.69	7.03	7.73	7.21	7.87	7.89	8.38
1	A	L	3	TRT	8.99	8.75	8.07	6.74	6.53	7.14	8.25
1	D	L	4	UNT	8.99	8.28	8.95	8.50	9.10	9.05	9.93
1	D	L	4	TRT	8.80	8.04	6.71	5.51	5.14	7.05	7.96

TRT A: RLD at dose duration D_1 (1.0 hour)
TRT B: RLD at dose duration D_2 (4.0 hours)
TRT C: Test drug product at dose duration of 2.0 hours
TRT D: RLD at dose duration of 2.0 hours
UNT: Untreated control site (no product applied) corresponding to each treated site

Table AIV.2 Baseline-adjusted a-scale data for a subject

SUB	TRT	ARM	LOC.	SITE	BL	Hours after drug removal					
						0	2	4	6	19	24
1	A	R	1	UNT	-	-0.11	0.75	0.30	0.48	0.34	1.37
1	A	R	1	TRT	-	0.75	0.48	-1.19	-0.88	-0.79	0.19
1	B	R	2	UNT	-	1.20	1.08	0.67	1.17	0.96	1.69
1	B	R	2	TRT	-	-0.50	-0.67	-2.34	-0.91	-0.78	0.47
1	C	R	3	UNT	-	1.04	1.52	0.49	1.47	0.31	1.27
1	C	R	3	TRT	-	1.48	1.52	-0.36	0.46	-0.38	0.81
1	D	R	4	UNT	-	0.88	1.30	0.25	1.57	0.21	0.82
1	D	R	4	TRT	-	0.84	-0.44	-2.31	-0.40	-0.14	0.53
1	C	L	1	UNT	-	0.36	-0.06	-0.14	-0.38	-0.89	-0.13
1	C	L	1	TRT	-	-1.55	-1.58	-2.58	-2.18	-1.21	-0.52
1	B	L	2	UNT	-	-0.80	-0.36	-0.87	-0.38	-0.32	0.46
1	B	L	2	TRT	-	-2.98	-3.08	-3.35	-3.07	-1.19	-0.21
1	A	L	3	UNT	-	-0.66	0.04	-0.48	0.18	0.20	0.69
1	A	L	3	TRT	-	-0.24	-0.92	-2.25	-2.46	-1.85	-0.74
1	D	L	4	UNT	-	-0.71	-0.04	-0.49	0.11	0.06	0.94
1	D	L	4	TRT	-	-0.76	-2.09	-3.29	-3.66	-1.75	-0.84

Table AIV.3 Baseline-adjusted, untreated control site-corrected a-scale data and $AUEC_{(0-24)}$ data for a subject

SUB	TRT	ARM	LOC.	SITE	BL	Hours after drug removal						$AUEC_{(0-24)}$
						0	2	4	6	19	24	
1	A	R	1	TRT	-	0.86	-0.27	-1.49	-1.36	-1.13	-1.18	-25.98
1	B	R	2	TRT	-	-1.70	-1.75	-3.01	-2.08	-1.74	-1.22	-45.53
1	C	R	3	TRT	-	0.44	0.00	-0.85	-1.01	-0.69	-0.46	-16.20
1	D	R	4	TRT	-	-0.04	-1.74	-2.56	-1.97	-0.35	-0.29	-27.29
1	C	L	1	TRT	-	-1.91	-1.52	-2.44	-1.80	-0.32	-0.39	-27.19
1	B	L	2	TRT	-	-2.18	-2.72	-2.48	-2.69	-0.87	-0.67	-42.26
1	A	L	3	TRT	-	0.42	-0.96	-1.77	-2.64	-2.05	-1.43	-46.87
1	D	L	4	TRT	-	-0.05	-2.05	-2.80	-3.77	-1.81	-1.78	-58.77

Table AIV.4 Baseline-adjusted, untreated control site-corrected a-scale data of all 12 subjects

TEST PRODUCT										REFERENCE PRODUCT									
				Hours after drug removal										Hours after drug removal					
SUB	TRT	ARM	LOC	0	2	4	6	19	24	SUB	TRT	ARM	LOC	0	2	4	6	19	24
1	C	R	3	0.44	0.00	-0.85	-1.01	-0.69	-0.46	1	D	R	4	-0.04	-1.74	-2.56	-1.97	-0.35	-0.29
1	C	L	1	-1.91	-1.52	-2.44	-1.80	-0.32	-0.39	1	D	L	4	-0.05	-2.05	-2.80	-3.77	-1.81	-1.78
2	C	R	3	-1.51	-3.29	-3.45	-4.11	-0.89	-1.26	2	D	R	4	-0.23	-1.58	-2.53	-2.53	0.00	-0.49
2	C	L	1	0.23	-1.09	-0.94	-2.15	-2.05	-0.66	2	D	L	4	-2.30	-2.88	-2.15	-3.05	2.09	0.27
3	C	L	3	-1.29	-1.75	-0.96	-0.90	-3.06	-1.05	3	D	R	1	1.25	-0.10	-1.99	-1.52	0.24	-1.24
3	C	R	2	0.02	-1.43	-2.24	-1.16	-1.56	-1.72	3	D	L	4	-0.04	-0.28	-1.30	-1.23	-0.77	-1.07
4	C	R	1	-0.02	-0.19	-0.52	-1.00	-0.43	-0.50	4	D	R	4	-0.43	-0.34	-1.50	-1.80	-0.74	-0.96
4	C	L	3	-0.12	0.15	-0.29	-0.06	-0.07	0.12	4	D	L	2	-0.47	-0.22	-0.49	-0.83	-0.89	-0.82
5	C	L	3	-0.36	-0.01	-0.19	-0.06	-0.72	-0.28	5	D	L	2	-0.71	-1.77	-1.62	-2.62	-0.76	-0.60
5	C	R	1	-0.02	-0.63	-1.13	-0.90	-0.88	-0.03	5	D	R	3	0.46	-1.23	-1.23	-1.61	-1.70	-0.47
6	C	R	4	0.60	0.32	0.32	0.30	-1.09	-1.53	6	D	R	1	-0.11	0.20	1.35	0.86	-0.77	-1.00
6	C	L	3	-1.08	-0.45	-0.98	-0.83	-1.18	-0.07	6	D	L	4	-0.95	-1.07	-0.52	-1.17	-2.33	-1.52
7	C	R	4	-0.28	0.25	-0.34	-0.64	-0.64	-0.41	7	D	R	2	-0.22	-0.30	-0.42	-0.18	-0.74	-1.00
7	C	L	1	0.67	0.74	0.72	1.03	0.33	-0.11	7	D	L	4	-0.51	0.03	-0.76	-0.12	-0.42	-1.24
8	C	R	4	-0.40	0.49	0.46	0.00	-0.35	0.78	8	D	R	2	0.51	0.30	0.92	0.63	0.56	0.34
8	C	L	3	0.30	0.05	0.07	0.19	-0.05	-0.28	8	D	L	1	-0.44	0.08	-0.16	-0.95	-2.00	-1.49
9	C	R	1	-0.71	-1.13	-1.94	-2.40	-1.70	-1.41	9	D	R	4	-0.40	-1.15	-2.25	-2.57	-1.20	-1.55
9	C	L	3	-0.34	-0.52	-1.46	-1.41	-0.31	-1.10	9	D	L	2	-1.16	-1.05	-1.90	-1.80	-1.06	-1.42
10	C	R	1	-0.49	-0.43	-0.63	-0.10	-0.50	-1.10	10	D	L	3	0.28	-0.31	-1.16	-1.40	-0.64	-0.57
10	C	L	3	0.10	-0.66	-0.44	-0.68	-0.34	-0.86	10	D	R	1	-0.14	-0.05	-0.24	-0.63	-0.41	-1.09
11	C	L	2	-0.58	-0.93	-1.60	-2.29	-0.24	-0.54	11	D	R	1	-0.46	-0.82	-1.10	-2.15	-0.47	-0.59
11	C	R	3	0.12	-1.67	-1.71	-2.34	0.15	-1.28	11	D	L	4	-0.15	-1.45	-1.66	-1.61	-1.14	0.55
12	C	L	3	0.05	-0.08	-0.18	-0.35	-1.28	-0.46	12	D	R	1	-0.25	-0.76	-1.35	-2.29	-1.23	-0.99
12	C	R	3	-0.60	0.15	0.19	-0.42	-0.40	-0.32	12	D	L	4	1.89	0.73	2.07	0.82	-0.59	0.70
MEAN				-0.30	-0.57	-0.86	-0.96	-0.76	-0.62					-0.19	-0.74	-1.06	-1.40	-0.71	-0.76
SD				0.65	0.91	1.01	1.12	0.76	0.59					0.79	0.87	1.23	1.2	0.9	0.68
SE				0.13	0.19	0.21	0.23	0.16	0.12					0.16	0.18	0.25	0.25	0.18	0.14
%CV				217	161	118	117	100	96					405	117	116	86	126	89

Table AIV.5 $AUEC_{(0-24)}$ data for the right arm, the left arm, and the two arm average at dose durations equal to D_1 and D_2, and the ratio of average AUEC at D_2/average AUEC at D_1 of all 12 subjects

$AUEC_{(0-24)}$ at D_1				$AUEC_{(0-24)}$ at D_2				AUEC at D_2/AUEC at D_1*
SUB	ARM	AUEC	AUEC (AVERAGE)	SUB	ARM	AUEC	AUEC (AVERAGE)	
1	R	-25.98	-36.42	1	R	-45.53	-43.90	1.21
1	L	-46.87		1	L	-42.26		
2	R	-62.43	-45.09	2	R	-69.72	-59.96	1.33
2	L	-27.76		2	L	-50.20		
3	R	-22.53	-28.41	3	R	-31.87	-64.04	2.25
3	L	-34.29		3	L	-96.21		
4	R	-7.49	-11.70	4	R	-27.48	-23.30	1.99
4	L	-15.91		4	L	-19.12		
5	L	-16.59	-17.36	5	L	-25.01	-16.58	0.95
5	R	-18.14		5	R	-8.15		
6	R	-8.24	-10.44	6	R	-27.36	-9.33	0.89
6	L	-12.64		6	L	8.70		
7	R	-10.89	-13.36	7	L	-20.44	-23.68	1.77
7	L	-15.83		7	R	-26.92		
8	L	7.08	4.69	8	R	-26.16	-21.02	-4.48
8	R	2.31		8	L	-15.88		
9	L	-34.22	-13.82	9	R	-33.80	-21.39	1.55
9	R	6.58		9	L	-8.97		
10	L	-4.10	3.06	10	R	-52.60	-43.79	-14.29
10	R	10.23		10	L	-34.97		
11	R	-33.30	-37.30	11	R	-57.00	-52.20	1.40
11	L	-41.30		11	L	-47.40		
12	R	-0.55	-21.06	12	R	-29.24	-28.22	1.34
12	L	-41.57		12	L	-27.20		

*Highlighted cells indicate AUEC ratio ≥ 1.25.

Table AIV.6 $AUEC_{(0\text{-}24)}$ data for right and left arms, the two arm average data for test and reference products of all 12 subjects at a dose duration of 2.0 hours

$AUEC_{(0\text{-}24)}$ of TEST PRODUCT					$AUEC_{(0\text{-}24)}$ of REFERENCE PRODUCT				
SUB	ARM	LOC	AUEC	AUEC* (AVERAGE)	SUB	ARM	LOC	AUEC	AUEC* (AVERAGE)
1	R	3	-16.20	-21.69	1	R	4	-27.29	-43.03
1	L	1	-27.19		1	L	4	-58.77	
2	L	3	-56.98	-48.52	2	R	4	-28.65	-22.20
2	R	1	-40.06		2	L	4	-15.75	
3	L	3	-43.63	-38.99	3	R	1	-15.27	-18.65
3	R	2	-34.36		3	L	4	-22.03	
4	R	1	-14.06	-7.62	4	R	4	-26.67	-22.42
4	L	3	-1.18		4	L	2	-18.18	
5	L	3	-8.39	-13.34	5	L	2	-35.48	-34.25
5	R	1	-18.29		5	R	3	-33.01	
6	R	4	-9.51	-15.23	6	R	1	0.01	-18.83
6	L	3	-20.96		6	L	4	-37.68	
7	R	4	-12.05	0.98	7	R	2	-12.17	-10.96
7	L	1	14.01		7	L	4	-9.75	
8	R	4	0.30	0.56	8	R	2	13.57	-7.94
8	L	3	0.81		8	L	1	-29.45	
9	R	1	-43.68	-32.05	9	R	4	-41.15	-37.40
9	L	3	-20.42		9	L	2	-33.65	
10	R	1	-10.61	-11.51	10	L	3	-20.35	-16.10
10	L	3	-12.41		10	R	1	-11.86	
11	L	2	-26.33	-26.18	11	R	1	-26.13	-26.73
11	R	3	-26.04		11	L	4	-27.33	
12	L	3	-15.77	-11.62	12	R	1	-35.19	-12.56
12	R	3	-7.47		12	L	4	10.08	
MEAN			-18.77	-18.77				-22.59	-22.59
SD			16.45	15.28				16.14	10.92
SE			3.36	3.12				3.30	2.23
%CV			88	81				71	48

*Highlighted cells show AUEC data of seven subjects whose AUEC ratios (see Table AIV.5) were ≥ 1.25, i.e., evaluable subjects. These AUEC data were used in the calculation of the 90% confidence interval in Appendix V.

APPENDIX V

LOCKE'S METHOD: FORMULAE AND A WORKED EXAMPLE

The calculation of the 90% confidence interval for the 'pivotal' bioequivalence data set of Table AIV.6 is given below. The data used to calculate the confidence interval are the average AUEC values of 'detectors' (evaluable subjects) only.

Table AV.1 Average AUEC values of subjects in the 'pivotal' study meeting the dose duration-response criterion of Section V(H)(1)(c)

Subject	$AUEC_{(0\text{-}24)}$ Test Product (Average)	$AUEC_{(0\text{-}24)}$ Reference Product (Average)
2	-48.52	-22.20
3	-38.99	-18.65
4	-7.62	-22.42
7	0.98	-10.96
9	-32.05	-37.40
11	-26.18	-26.73
12	-11.62	-12.56

The calculation of the confidence interval is facilitated by the calculation of the following intermediate quantities:

$$\bar{X}_T = \frac{1}{n}\sum_{i=1}^{n} X_{Ti} \qquad \bar{X}_R = \frac{1}{n}\sum_{i=1}^{n} X_{Ri}$$

where n is the number of evaluable subjects, seven (7) in this example.

$$\hat{\sigma}_{TT} = \frac{\sum_{i=1}^{n} (X_{Ti} - \bar{X}_T)^2}{n - 1} \qquad \hat{\sigma}_{RR} = \frac{\sum_{i=1}^{n} (X_{Ri} - \bar{X}_R)^2}{n - 1}$$

$$\hat{\sigma}_{TR} = \frac{\sum_{i=1}^{n} (X_{Ti} - \bar{X}_T)(X_{Ri} - \bar{X}_R)}{n - 1}$$

These are the sample means, sample variances, and sample covariance for the individual evaluable subject average AUEC data. For the example, these are

$$\bar{X}_T = -23.43 \quad \bar{X}_R = -21.56 \quad \hat{\sigma}_{TT} = 323.13 \quad \hat{\sigma}_{RR} = 80.10 \quad \hat{\sigma}_{TR} = 78.83$$

Define t as the 95th percentile of the t-distribution for n-1 degrees of freedom. For example, for n = 7, t (6 degrees of freedom) is 1.9432. Now define

$$G = \frac{t^2 \, \hat{\sigma}_{RR}}{n \, \bar{X}_R^{\,2}}$$

$G < 1$ is required to have a proper confidence interval. If $G \geq 1$, the study does not meet the *in vivo* bioequivalence requirements. In the example, G = .0930.

Under the assumption that $G < 1$, calculate

$$K = \left(\frac{\bar{X}_T}{\bar{X}_R}\right)^2 + \frac{\hat{\sigma}_{TT}}{\hat{\sigma}_{RR}}(1 - G) + \frac{\hat{\sigma}_{TR}}{\hat{\sigma}_{RR}}\left(G\frac{\hat{\sigma}_{TR}}{\hat{\sigma}_{RR}} - 2\frac{\bar{X}_T}{\bar{X}_R}\right)$$

In the example, K = 2.791.

The confidence interval limits may now be calculated:

$$\frac{\left(\frac{\bar{X}_T}{\bar{X}_R} - G\frac{\hat{\sigma}_{TR}}{\hat{\sigma}_{RR}}\right) \mp \frac{t}{\bar{X}_R}\sqrt{\frac{\hat{\sigma}_{RR}}{n} K}}{1 - G}$$

In the example, 90% confidence interval limits are 53.6% and 165.9%, based on the data of seven evaluable subjects.